Liposome Immobilization on Peptide-modified Quartz Crystal Microbalance Electrodes for Kinetic Analysis of Interactions on Membrane Surfaces

Yuzo Kasuya, Megumi Ohtaka, Kei Tsukamoto, Yasuyuki Ikeda, and Kazunari Matsumura*
Department of Materials Science and Engineering, Faculty of Engineering, Shibaura Institute of Technology, 3-7-5 Toyosu, Koto-ku, Tokyo 135-8548

(Received February 25, 2008; CL-080211; E-mail: matumura@sic.shibaura-it.ac.jp)

In order to facilitate biosensor analyses of interactions on membrane surfaces, a method of immobilizing liposome using anchoring amphiphilic peptides was developed. Intact liposomes were immobilized stably on a quartz crystal microbalance electrode and were applied to a quantitative study of glycolipids–protein interactions.

Many of biomolecular interactions which are crucial for cell processes occur at membrane surfaces. Since most membrane-bound molecules are likely to have different structures and binding affinities in solution, it is desirable to immobilize them onto analytical devices together with reconstituted membrane systems for investigating these interactions. Supporting planer lipid membranes onto solid surfaces yields stable membrane systems reproducibly,[1] but these films have less fluidity and cannot mimic perfectly plasma environments. Therefore immobilization of intact liposomes on sensor surfaces has attracted much attention. The modifications of both liposomes and sensors chip for assembling them with intermolecular interaction, such as histidine tag–nickel chelate,[2] biotin–avidin complex,[3] DNA duplex[4] and antibodies–antigen complex,[5] have been reported. Although lipophilic anchors attached to hydrogel are widely used for immobilizing liposomes due to unnecessity of tethered lipids,[6] detailed kinetic studies have been scarcely reported. Transformation to a planer bilayer at a critical coverage limits both the sensitivity and reproducibility.[7] To prepare a stable liposomes adlayer without fusing membranes, we have proposed an immobilizing method based on small-peptides instead of polymeric anchors.[8] Herein we present the immobilization of intact liposomes onto the Au layer of a quartz crystal microbalance (QCM) and its analytical application to the detection of protein–carbohydrate interactions.

The preparation of a liposomal QCM sensor electrode followed the procedure depicted in Figure 1. A cleaned Au electrode was exposed to a HEPES-buffered 1 μM solution of the synthetic anchor peptide (K9WA5C: KKKKKKKKKW-AAAAAC), which contains an amphiphilic segment with a cysteine residue for Au-binding. Adsorption mass changes of the electrode were monitored by QCM equipped with an AT-cut 27 MHz quartz plate. Throughout all measurements in this work, the surface densities of bound peptides were adjusted to 0.67 pmol/mm^2 by the addition of an appropriate amount of peptide solutions.

To assess liposome binding by QCM, the peptide-modified electrode was cleaned with 50 mM HEPES buffer, before an aliquot of 100-nm liposomes containing phosphatidylcholine (PC), phosphatidylserine (PS) and cholesterol (Chol) was added.[9] The maximum frequency shift showed that the K9WA5C peptides immobilized liposome at a density of

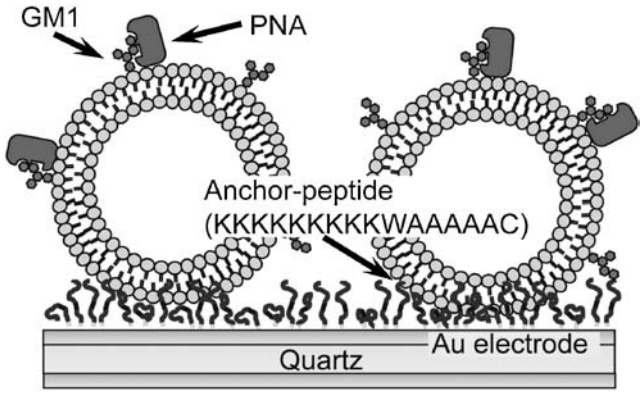

Figure 1. Schematic illustration of the liposome-immobilized QCM sensor probe: Au electrode surface prepared by (i) adsorption of K9WA5C via Au/thiol contact and (ii) immobilization of liposomes which contains 10 mol % glycolipids, followed by (iii) addition of lectin to detect the interaction.

22 ng/mm^2. This surface density, which is more than five times the amount of material expected for formation of a planar lipid bilayer (ca. 4 ng/mm^2), includes the weight of a solution encapsulated by the intact liposomes. The significant mass decrease by treatment with detergent (Triton X-100) which disrupts liposomes was also observed. The absorbed mass was reduced by 80–85% using variant sequences in which the Lys segment was truncated or the Trp position was altered.[8] Interestingly, desorption of liposome from the saturated adlayer was not detected for buffer exchanges while the initial adsorption processes could be satisfactorily described by simple Langmuir adsorption kinetics.

Nonruptured liposomes on the peptide-modified Au substrate were observed directly with atomic force microscopy.[9] K9WA5C-modified surfaces kept liposomes intact at considerably higher adsorption coverage than a glass substrate as one of the hydrophilic surfaces. These results showed that the Au surface is not hydrophilic enough to induce membrane fusion despite amphiphilic modification, unlike hydrophilic surfaces and polymers which are known to induce fusion of liposomes.[7b,10] In addition, the penetration of K9WA5C into liposomes is shallow because Lys segments prefer to be located close to the aqueous phase and the Trp side chain is located near the lipid carbonyl region[11] whereas fusogenic protein produces a large hydrophobic perturbation which would lead to fusion.[12]

To validate our approach, we measured the well-studied interactions of lectins with monosialoganglioside GM1 (GM1) and asialoganglioside GM1 (GA1) in immobilized liposomes using K9WA5C. The stepwise frequency shift as a result of binding between GM1 and peanut agglutinin (PNA), which has a binding

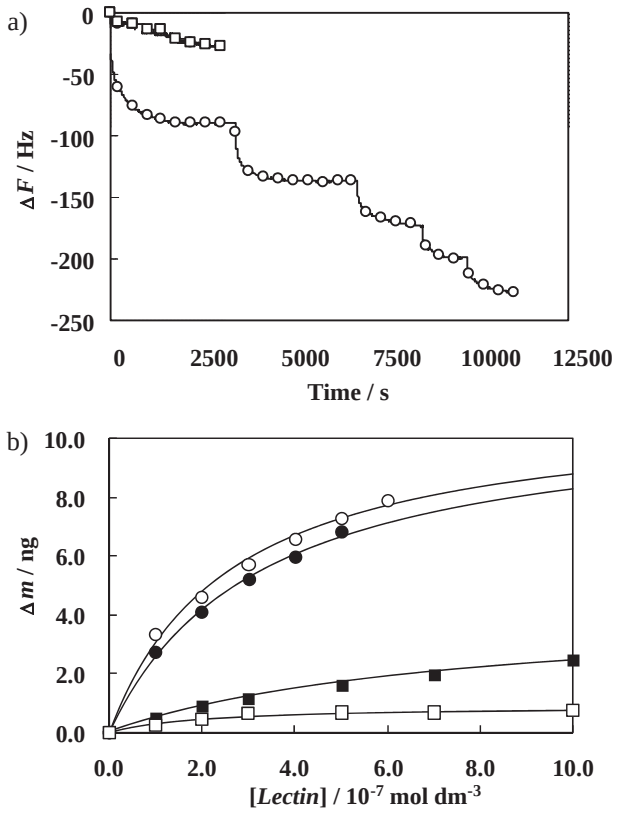

Figure 2. a) Typical time courses of frequency changes of a 27 MHz quartz plate with GM1-doped liposome-immobilized electrode upon a stepwise addition of lectin to yield a 1 μM solution. Symbols: PNA (circle) and LcH (square). b) The binding isotherm of PNA (circuler) and WGA (square) to GM1 (closed mark) or GA1 (open mark) on liposomes in HEPES buffer at 37 °C.

site for disaccharide Gal(β1-3)GalNAc of GM1, was observed whereas the frequency was hardly decreased by using lens culinaris hemagglutinin (LcH) which shows absence of interaction with GM1 (Figure 2a). The time courses were consistent over several hours without irregular drifts caused by agglutination.

The glycolipid–lectin binding constants (K_a) and the maximum binding amounts (Δm_{max}) were obtained from the binding isotherms of PNA and wheat germ agglutinin (WGA) (Figure 2b). For GM1–PNA interaction $\Delta m_{max} = 2.3 \pm 0.1$ ng/mm^2 and $K_a = 3.2 \pm 0.4 \times 10^6$ M^{-1} were obtained, while for GA1–PNA, K_a reads $4.1 \pm 0.3 \times 10^6$ M^{-1} and $\Delta m_{max} = 2.3 \pm 0.2$ ng/mm^2. The higher binding constant of PNA to GA1 demonstrates that the sialic acid (NeuAc) linked to GM1 impairs the binding. The equality of both Δm_{max} shares similarity with the findings obtained from liposomal affinity chromatography[13] rather than those from QCM study of a hybrid bilayer composed of octanethiol and lipids/GM1 on electrodes.[14] For WGA as ligand to GM1, we obtained values of $K_a = 1.2 \pm 0.2 \times 10^6$ M^{-1} and $\Delta m_{max} = 0.8 \pm 0.1$ ng/mm^2, which was a third of Δm_{max} for PNA, corresponding to the molecular weight of WGA.[15] The relatively low affinity of WGA has been reported. The significant small Δm_{max} for WGA–GA1 shows that the binding target of WGA is a sialic acid. All the binding constants and binding amounts, as observed above, were fairly consistent with each other and showed the effectiveness of our approach.

Here we have demonstrated that our facile method based on small peptides is applicable to determination of the kinetics of glycolipid–lectin interactions. There are two advantages to use this liposome-immobilizing approach: (1) there is no need to dope tethered lipid to liposomes, though (2) the liposomes can be captured without fusing at high enough densities to allow the reliable study of biomembrane interaction. Moreover, it is a great advantage utilizing the liposomal sensor in the analysis of ligand binding to a transmembrane receptor. The quantitative evaluation of the difference between the lectin–glycolipid affinity on intact liposomes and those on the supported hybrid bilayer is now in progress.

The authors are grateful to Prof. M. Komiyama (Tokyo University) for the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis and the helpful discussion.

References and Notes

1 a) A. Granéli, J. Rydström, B. Kasemo, F. Höök, *Langmuir* **2003**, *19*, 842. b) P. Stenlund, G. J. Babcock, J. Sodroski, D. G. Myszka, *Anal. Biochem.* **2003**, *316*, 243.
2 T. Stora, Z. Dienes, H. Vogel, C. Duschl, *Langmuir* **2000**, *16*, 5471.
3 a) Q. Yang, X.-Y. Liu, S. Ajiki, M. Hara, P. Lundahl, J. Miyake, *J. Chromatogr. B* **1998**, *707*, 131. b) S. Morita, M. Nukui, R. Kuboi, *J. Colloid Interface Sci.* **2006**, *298*, 672.
4 C. Yoshina-Ishii, G. P. Miller, M. L. Kraft, E. T. Kool, S. G. Boxer, *J. Am. Chem. Soc.* **2005**, *127*, 1356.
5 C. R. MacKenzie, T. Hirama, K. K. Lee, E. Altman, N. M. Young, *J. Biol. Chem.* **1997**, *272*, 5533.
6 a) M. A. Cooper, A. Hansson, S. Löfås, D. H. Williams, *Anal. Biochem.* **2000**, *277*, 196. b) For a review, see: M. Beseničar, P. Maček, J. H. Lakey, G. Anderluh, *Chem. Phys. Lipids* **2006**, *141*, 169.
7 a) E.-M. Erb, X. Chen, S. Allen, C. J. Roberts, S. J. Tendler, M. C. Davies, S. Forsen, *Anal. Biochem.* **2000**, *280*, 29. b) E. Reimhult, F. Höök, B. Kasemo, *Langmuir* **2003**, *19*, 1681.
8 For details of various small peptides immobilizing liposomes, see: Y. Kasuya, M. Ohtaka, K. Tsukamoto, Y. Ikeda, K. Matsumura, *Peptide Sci.* **2007**, in press.
9 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
10 D. Meyuhas, S. Nir, D. Lichtenberg, *Biophys. J.* **1996**, *71*, 2602.
11 M. R. R. Planque, J. A. W. Kruijtzer, R. M. J. Liskamp, D. Marshi, D. V. Greathouse, R. E. Koeppe, B. Kruijff, J. A. Killian, *J. Biol. Chem.* **1999**, *274*, 20839.
12 M. Gasset, M. Onaderra, P. G. Thomas, J. G. Gavilanes, *Biochem. J.* **1990**, *265*, 815.
13 J.-E. Månsson, S. Olofsson, *FEBS Lett.* **1983**, *156*, 249.
14 C. Steinem, A. Janshoff, J. Wegener, W.-P. Ultrich, W. Willenbrink, M. Sieber, H.-J. Galla, *Biosens. Bioelectron.* **1997**, *12*, 787.
15 T. Sato, T. Serizawa, F. Ohtake, M. Nakamura, T. Terabayashi, Y. Kawanishi, Y. Okahata, *Biochim. Biophys. Acta* **1998**, *1380*, 82.

Published on the web (Advance View) April 26, 2008; doi:10.1246/cl.2008.588